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NATURAL HYBRIDIZATION IN *CENTAURIUM ERYTHRAEA* AND *C. LITTORALE* AT FRESHFIELD AND AINSDALE, SOUTH LANCASHIRE

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ABSTRACT

Nine populations of *Centaurium* were studied at Freshfield and Ainsdale, near Southport, Lancashire, England. The two species *C. erythraea* Rafin. and *C. littorale* (D. Turner) Gilmour flowered during the same time and were found growing sympatrically in some populations. Cytological studies revealed that both species had approximately the same range of variation in their chromosome number ; *C. erythraea* had $n=7$, 8, 9 and 10 while *C. littorale* had $n=8$, 9, 10 and 11. These chromosome numbers are new records for these species. Breeding experiments showed that both the species were self-compatible and largely autogamous but were capable of cross-pollination. Large populations of hybrids were seen at Freshfield. These hybrids were morphologically distinct and almost exactly intermediate between the two species. The hybrids were analysed by the hybrid index method of Anderson (1936) and inspite of being fertile did not show any great variability among themselves and probably represent an F. 1 generation. Although *C. erythraea* and *C. littorale* were sympatric in several different kinds of habitat it was only in *Pinus nigra* plantations that the hybrids became established.

INTRODUCTION

The two species of *Centaurium*, *C. erythraea* Rafin. and *C. littorale* (D. Turner) Gilmour are highly polymorphic. This polymorphism has been noticed by several workers cited by Robyns (1954) and a number of varieties in each of the species were described by Wheldon & Salmon (1925). Frequently some of the varieties of one species fall within the specific limits of the other or could be classed as intermediates.

The occasional occurrence of hybrids between the two species has been reported in Britain by Clapham, Tutin and Warburg (1962). Their occurrence in Freshfield, near Southport, Lancashire, has been reported by O'Connor (1955) and Savidge, Heywood and Gordon (1963). During the summer of 1967 at Freshfield the two species *C. erythraea* and *C. littorale* have been seen growing sympatrically as well as in separate populations. Populations of intermediates which appeared to be hybrids were noticed in the vicinity of *Pinus nigra* woodlands. During the course of present studies, morphology, breeding behaviour and taxonomy of both the species as well as their hybrids was studied. Cytology has also been studied in both these species and is briefly described in this note.

MORPHOLOGY

Altogether nine populations were studied. Six populations at Freshfield and three at a site near by at Ainsdale. When the flowers started opening in the first week of July, a sample of about forty plants was gathered from each of these populations and brought to the laboratory. The flower colour of each of the specimens was noted. One or two flowers from each specimen were preserved in alcohol for pollen measurements. All other measurements were made on dried and pressed specimens.

The plants gathered from various populations fell into three distinct categories which were represented in various proportions in the populations as follows:

1. *C. erythraea*
2. *C. littorale*
3. *hybrids*

The hybrids were intermediate between the two species in stem height, leaf length/breadth, number of flower buds and the colour of flowers. Histograms for stem height and leaf length/breadth representing three different populations are illustrated in fig. 1. The range of leaf length/breadth and stem height of plants in the populations are given below:

Name of species	*Leaf length/breadth*	*Stem height*
C. erythraea	1.5—3.5	11—31 cm
C. littorale	3.5—8.5	5—11 cm
hybrids	2.5—6.5	9—23 cm

CYTOLOGY

Meiosis was studied in *C. erythraea* and *C. littorale*. Young flower buds were fixed in 1 : 3 acetic alcohol and were transferred to 70% alcohol after

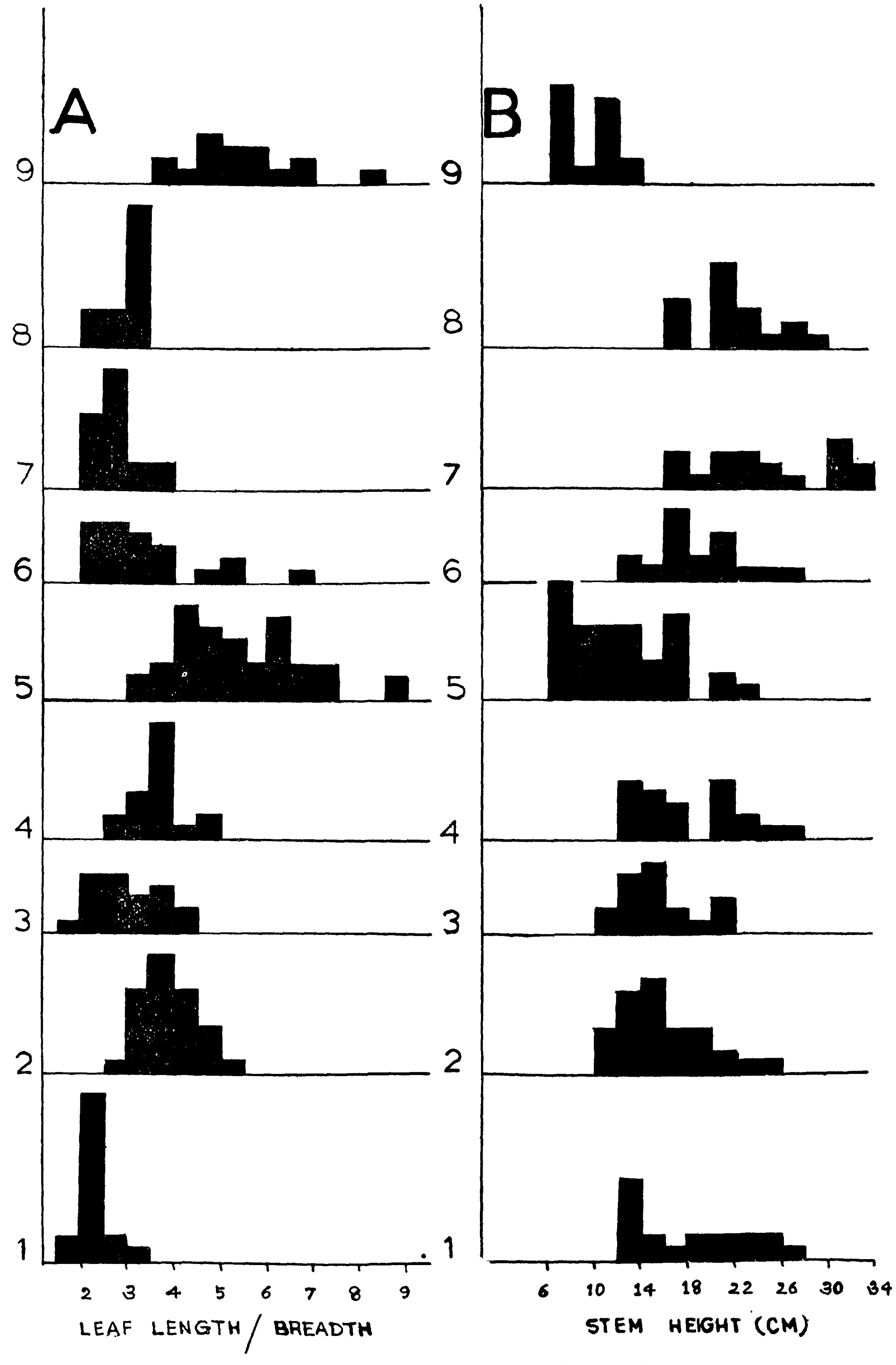

Fig. 1. A. Histograms of leaf length/breadth. B. Histograms of stem height, representing 3 different populations i.e. *Centaurium erythraea*, *C. littorale* and the hybrids among the 9 populations studied. It is clear that except for populations 7 and 8, which are pure *C. erythraea* populations, the hybrids are present in all others.

24 hours and stored in a deep freeze. Acetocarmine squashes were made to study the chromosomes.

Wulff (1937) was the first person to study the cytology of genus *Centaurium*. He studied two species *C. littorale* and *C. pulchellum* and reported $n=19$ in each. He however stated that his counts were approximate. Rork (1949) studied the chromosome numbers in family Gentianaceae reported $2n=42$ in *C. erythraea*. Zeltner (1962) reported $n=20$ in both *C. erythraea* and *C. littorale*. In the Freshfield and Ainsdale plants, however, chromosome counts of $n=7$, 8, 9, 10 were obtained in *C. erythraea* and $n=8$, 9, 10, 11 were obtained for *C. littorale* (Fig. 2). Both these are new reports for the two species.

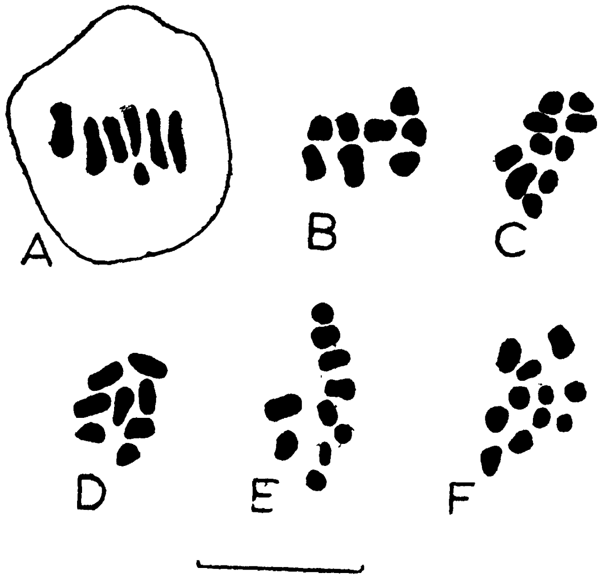

Fig. 2. Chromosome numbers of *Centaurium erythraea:* (A) $n=7$; (B) $n=8$; (C) $n=10$. Chromosome numbers in *C. littorale* : (D) $n=8$; (E) $n=10$; (F) $n=11$.

BREEDING SYSTEM

Breeding experiments for each of the species and their hybrids were set up in Freshfield. Three experiments were set up for each of these taxa. First to study compatibility, second to study cross-fertilization in the same species and third to investigate agamospermous mechanisms. Interspecific crosses were also made between the two species. Ten plants were selected for each of the experiments and seed set was examined after a month.

Methods:

Expt. I. Ten plants of each of the taxa were covered with pollen proof bags made of nylon net to see if they were self-fertile. Since anthesis takes place before the flowers fully open, only inflorescences with unopened flowers were covered.

Expt. II. Plants were cross-pollinated artificially. Stamens were uncovered from unopened buds and were removed with forceps. Buds in which even a single stamen had dehisced were rejected. The flowers and buds which were not to be pollinated were removed by hand. Stamens removed from a nearby plant were then rubbed on the stigma of the emasculated flowers. The flowers pollinated were then covered with bags made of nylon net.

Expt. III. Unopened buds were emasculated and covered with nylon nets to see if there was any possibility of apomixis.

Results: *Expt. I*

Name of species	No. of buds covered	No. of fruits developed	% of fruit developed
C. erythraea	240	240	100
C. littorale	50	40	80
hybrids	308	308	100
	Expt. II		
C. erythraea	18	16	89
C. littorale	16	14	87
hybrids	16	12	75

Expt. III

In Expt. III none of the emasculated buds produced fruit indicating that agamospermy is unlikely.

INTERSPECIFIC CROSSES

Field No. of plant	Female parent cited first Crosses	No. of fls. pollinated	No. of fruits developed	% of fruit developed
126	*C. erythraea* × *littorale*	3	2	60
127	*C. erythraea* × *littorale*	3	3	100
128	*C. erythraea* × *littorale*	3	3	100
129	*C. erythraea* × *littorale*	3	0	0
130	*C. erythraea* × *littorale*	3	3	100
131	*C. littorale* × *erythraea*	5	5	100

HYBRIDIZATION

In four populations which contained hybrids, a hybrid index was calculated based on the method devised by Anderson (1936) and adapted by Bradshaw (1958). The following six characters were chosen to differentiate the two species.

C. erythraea	*C. littorale*
1. Bracts and upper leaves glabrous.	1. Bracts and upper leaves scabrous-papillose at margin and main veins.
2. Basal leaves obovate-oblong.	2. Basal leaves linear or linear-lanceolate.
3. Stem leaves ovate or ovate-oblong.	3. Stem leaves linear.
4. Cymes compact, dense.	4. Cymes lax.
5. Calyx glabrous.	5. Calyx scabrous-papillose.
6. Calyx at anthesis 3-4 mm long, reaching half way to 2/3 the length of corolla tube.	6. Calyx 5-8 mm long, reaching the top of corolla tube or beyond.

Each character for *C. erythraea* scored 0 and that for *C. littorale* scored 4. Exact intermediates scored 2. The characters which were not exact intermediates but were more like *C. erythraea* scored 1 and those which were more like *C. littorale* scored 3. Index values for all the characters were added together for each plant. The index value for *C. erythraea* was 0 and for *C. littorale* 24. The plants with index values 1-4 were considered *C. erythraea*. Those with index values 20-24 as *C. littorale* and the rest as hybrids. For each population 40 plants were scored. The histograms of index values against frequency are given in fig. 3. It is clear from the histograms that in all the populations hybrids form almost exact intermediates between the parent species and that introgession in the Andersonian sense is not taking place.

TAXONOMY

Distinguishing features between *C. littorale*, *C. erythraea* and the hybrids as seen in Freshfield plants are given below in table I.

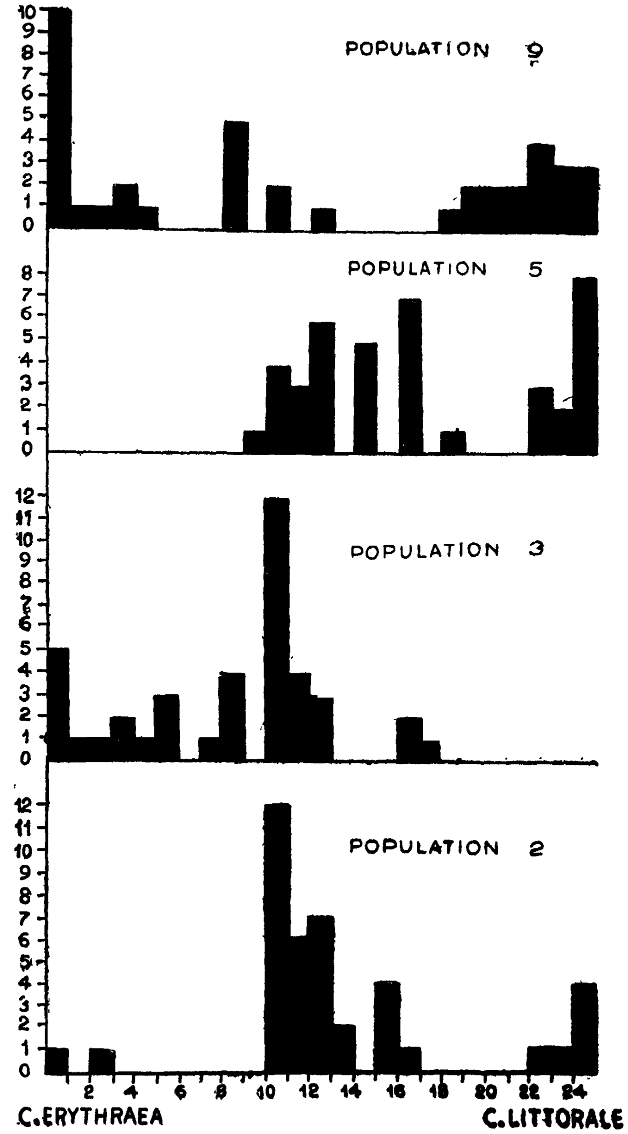

Fig. 3. Histograms of Index Values of *Centaurium erythraea*, *C. littorale* and their hybrids, indicating that hybrids form almost exact intermediates between the parent species and that introgression in the Andersonian sense is not taking place.

TABLE I

C. erythraea	*Hybrids*	*C. littorale*
1. Stem 11-32 cm high.	1. Stem 9-26 cm high.	1. Stem 5-12 cm high.
2. Simple or branched from base or above.	2. Unbranched or branches arising from base.	2. Unbranched or branches arising from base.
3. Leaves glabrous, obtuse or acute.	3. Margins and main veins especially in the upper leaves scabrous-papillose, obtuse or acute.	3. Margins and main veins especially in the upper leaves scabrous-papillose, obtuse.
4. Basal leaves obovate-spathulate or obovate-oblong.	4. Basal leaves obovate-spathulate to obovate-lanceolate.	4. Basal leaves linear-lanceolate to linear-spathulate.
5. Stem leaves ovate, ovate-oblong to elliptic-oblong.	5. Stem leaves elliptic-lanceolate.	5. Stem leaves linear to linear-lanceolate.
6. Flowers numerous in compact corymbose cymes.	6. Flowers numerous in compact or lax corymbose cymes.	6. Flowers in lax few-flowered fastigiate cymes.
7. Corolla rose or white.	7. Corolla rose above, deep rose on the back surface.	7. Corolla deep rose.
8. Calyx glabrous.	8. Calyx densely papillose-scabrous.	8. Calyx densely papillose-scabrous.
9. Calyx at anthesis 3-4 mm long.	9. Calyx at anthesis 5-8 mm long.	9. Calyx at anthesis 5-8 mm long.

DISCUSSION

Cytology: The cytological studies of the Freshfield plants reveal that both the species *C. erythraea* and *C. littorale* have chromosome numbers within approximately the same range of variation. In *C. erythraea* the chromosome numbers were $n=7$, 8, 9 and 10. Zeltner (1962) considers $n=10$ as the chief basic chromosome number in genus *Centaurium*. With the exception of *C. spicatum* which has $x=11$, all the species of *Centaurium* investigated by Zeltner (1961, 1962, 1963, 1966) have $x=10$ as the basic chromosome number. *C. pulchellum* in addition to having $x=10$ also has $x=7$ and $x=9$ as basic chromosome numbers (*Khoshoo*, 1966).

Zeltner (1963) has reported $n=20$ in *C. littorale*; that is the only report available so far on this species. He further considers this species to be a tetraploid. The Freshfield plants of this species which have chromosome numbers $n=8$, 9, 10, 11 perhaps represent diploids with some degree of aneuploidy. The reports of Wulff (1937) $2n=38$ and Warburg in Maude (1939) $2n=56$ do not agree with the results obtained in the present studies and with those of Zeltner (1963). But Wulff (1937) has stated that his results are approximate.

The cytology of *C. erythraea* is rather difficult because the chromosomes have a tendency to overlap and do not spread properly. The same difficulty has been encountered by Zeltner (1962). In this species the Freshfield plants have chromosome numbers $n-7$, 8, 9, 10, whereas the previous count by Zeltner (1962) is $n=20$. In this species the Freshfield plants also represent a diploid race with some aneuploidy whereas Zeltner's plants are tetraploids. These results are contradictory to those of Rork (1949) who found $2n=42$ in *C. erythraea*. It is probable that his plants belong to a different race with basic number $x=7$. The same basic number has been reported by Khoshoo (1966) in one of the Indian races of *C. pulchellum*. Rork (1949) describes $x=5$, 7, 9 and 13, basic numbers in Gentianaceae as a whole.

If after Zeltner (1962) $x=10$ is accepted as the basic number in *Centaurium*, both the species *C. littorale* and *C. erythraea* have polyploid races. Polyploid races are also present in *C. tenuiflorum* and *C. pulchellum*. Out of these four, three species *C. littorale*, *C. erythraea* and *C. pulchellum* have been considered very variable (*Robyns* 1954).

Breeding system: From the results of breeding experiments it is evident that *C. erythraea*, *C. littorale*, and their hybrids are capable of self and cross-pollination. The two species also produce fertile hybrid progeny. However, it appears that it is the self-pollination which occurs most frequently in these taxa because anthesis takes place before the flowers are fully open and pollen can be seen deposited on the stigma and the base of the petals. Moreover, at anthesis the tips of the stamens become slightly curved towards the stigma. Anthers which get detached at the base and remain attached at the apex, get coiled up so that they can easily touch the stigma.

These results are, however, not in agreement with those of Gilmour (1934) and O'Connor (1955) who maintain that *C. erythraea* is mostly self-pollinated. These authors, however, have not given any experimental evidence to support their views. The possibility of the occurrence of a certain degree of cross-pollination cannot, however, be discounted in either species. There is more probability of *C. littorale* being self-pollinated, firstly because the populations of this species usually consist of smaller numbers of individuals and secondly no obvious morphological variation was noticed within the populations. The occurrence of one or two specially isolated individuals is not uncommon in this species. Since all the isolated individuals were seen setting seed and almost all the flowers developed mature fruits it is more likely that they are self-pollinated.

Hybridization: Both *C. erythraea* and *C. littorale* have chromosome numbers in approximately the same range of variation and they flower at the same time. Since both the species grow largely sympatrically they are likely to hybridize but the hybrids will only become established in a suitable environment.

Hybridizaion seems to be common in genus *Centaurium*. *C. tenuiflorum* (Hoffmanns & Link.) Fritsch. and *C. latifolium* (Sm.) Druce are considered by Melderis (1932) and Wittrock (1884) to be hybrids between *C. erythraea* and *C. pulchellum*. Fertile artificial hybrids between *C. erythraea* and *C. littorale* have also been raised by Melderis.

Among hybrids between *C. erythraea* and *C. littorale* very little variation in morphological features has been noticed and they all appear to be exact intermediates. Very few plants which fall between exact intermediates and either of the parents have been noticed. This probably means that these hybrids do not appear to go further than F_1 generation in the field and that very few if any F_2 or back crosses get established. This may be the reason that the two species have retained their specific entities. Similar phenomena have been noticed by Epling (1947) in *Salvia apiana* and *S. mellifera* and by Stebbins, Matske & Epling (1947) in *Quercus marilandica* and *Q. ilicifolia.* The reason that very few back crosses or F_2 plants have been formed may lie in comparative sterility or inviability of F_2 and back crosses or, perhaps, because they are not favoured by natural selection.

The F_1 hybrids between the two species were produced experimentally by Melderis (1932). Freshfield hybrids agree with the description of F_1 hybrids given by Melderis in most of the characters except for the colour of the corolla lobes which is deep rose in Melderis's hybrids while in Freshfield hybrids it is rose on the upper surface and deep rose on the lower surface. But these differences may be due to the fact that the Freshfield plants represent different genotypes as is evident from the difference in chromosome number. Bradshaw (1958) has mentioned that it is difficult to know the range of variation of the F_1 hybrids because experimental material may not behave in the same way as the natural populations. Jones (1956) and Stebbins (1945) have stated that very different F_1 hybrids can be produced when different genotypes of the same species are used as parents in a particular cross.

The frequent association of a few plants of *C. littorale* with hybrid populations indicates that this species at Freshfield may act as the female parent and that the seeds thus produced are scattered around them.

The hybrids have been noticed only in the vicinity of *Pinus nigra* woodlands. As one goes farther away from the trees of *Pinus nigra* their number gets considerably reduced or they are altogether absent and are replaced by *C. erythraea.* It appears that hybrids have become established due to the new environment created by *Pinus nigra* woodlands which were planted about fifty to sixty years ago and prefer undisturbed open land in their vicinity. Along the Fisherman's path in the woodland and on open dunes where there is considerable disturbance, hybrids are absent and are replaced by *C. erythraea.* In the Ainsdale Sand dunes where the conditions are just as rigorous but no pines have been planted the hybrids are absent and it is *C. erythraea* which occurs there.

Taxonomy: Both the species are polymorphic. A number of varieties have been described by Wheldon & Salmon (1925). Some of these varieties are of intermediate nature between the two species. Thus *C. erythraea* var. *sublittorale* Wheld. & Salm., which shows some resemblances to *C. littorale* in its narrower leaves, richly coloured corolla and slightly scabrid leaves, is considered by its authors as a hybrid between *C. littorale* var. *occidentale* Wheld. & Salm., and *C. erythraea.* On the other hand, *C. littorale* var. *minus* (Hartm.) Gilmour seems to be closely related to *C. erythraea* because of its glabrous stem, spathulate basal leaves, flowers in compact cymes and smooth or slightly scabrid calyx.

Schmidt (1832), because of these intermediate forms, united both the species into a single species *Erythraea centaurium* Rich and subdivided it into two subspecies subsp. *vulgaris* Schmidt and subsp. *linarifolia* Schmidt. but subsequent authors, such as, Grisebach (1845), Gilg (1895), Wheldon & Salmon (1925), Hegi (1927) and Gilmour (1937) did not agree with him and retained the two species as separate entities.

Among the previously described taxa the Freshfield hybrids most closely resemble *C. erythraea* var. *fasciculare* (Duby) Robyns but are obviously different after a comparison with the two sheets of this variety present in University of Liverpool Herbarium (LIVU). The herbarium sheets had glabrous calyx, leaves and bracts, whereas in hybrids they were scabrous-papillose especially at the margins. Moreover Gilmour in his description of this variety mentions calyx as glabrous.

The hybrids resemble *C. littorale* in the length of calyx lobes which are 5-8 mm, calyx scabrous-papillose at margins and main veins of bracts and upper leaves scabrous-papillose. The characters resembling *C. erythraea* are the habit, height of the plant, basal leaves which are obovate-oblong and inflorescence which is compact corymbose cyme. The stem leaves are elliptic-lanceolate and are intermediate between the two species. Corolla colour is also intermediate; it is white or rose in *C. erythraea* and deep rose in *C. littorale,* whereas in hybrids it is deep rose on the lower surface and rose on the upper surface. Thus the hybrids form a more or less distinct but transitional group between the two species.

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